

Endosymbiotic Bacteria within *Bryopsis* species (Bryopsidales, Chlorophyta): Naming the Actors

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Microbial symbionts have been well documented in animals and plants, but they are also commonly found in association with various algal groups. In the marine green alga *Bryopsis* the endosymbiotic bacteria even seem to be present in every stage of the life cycle, suggesting vertical transmission of the endosymbionts¹. This indicates an ancient association among host and symbiont, rather than a recent opportunistic and non-specific relationship. Although this remarkable algal-bacterial partnership was already noticed in the early 1970s, no research has been performed to explore the physiological nature and specificity of the endosymbiosis. Therefore this research focuses on the identity and diversity of endosymbiotic bacteria within *Bryopsis* species. To identify the bacterial partner, epiphytes were chemically and enzymatically removed from *Bryopsis* plants from diverse geographical regions. Incubation of sterilized *Bryopsis* samples on Marine Agar plates showed no bacterial growth. Moreover, staining of the sterilized thalli with DAPI revealed the absence of nearly all bacterial fluorescence on the surface of the algae. The algal cells themselves were not lysed, suggesting the endophytic bacteria might still be present within the *Bryopsis* plants after sterilization. Subsequently, the different samples were submitted to a range of molecular techniques such as 16S rDNA PCR, cloning, DGGE and DNA sequencing. The phylogenetic analysis revealed that only a small fraction of clones carried bacterial sequences, in contrast to the majority of the clones which had the *Bryopsis* 16S rRNA chloroplast gene inserted. The obtained bacterial sequences covered just five different species: (i) two diverse species belonging to the Rhizobiales, (ii) a Bacteroidetes bacterium (iii) a bacterium in tight phylogenetic alliance with *Labrenzia alba*, and (iv) a species closely related to the *Rickettsia* symbiont of a marine organism. All five bacteria are present in several *Bryopsis* plants from various regions. Not only does this indicate that these endophytic bacteria are preserved within *Bryopsis* species, it also suggests that they might be actual endosymbionts with a significant function. The occurrence of bacteria belonging to the Bacteroidetes, Rhizobiales and Rickettsiales, with well-known symbiotic features, validates this hypothesis. Attempts are being made to visualize the endosymbionts with electron microscopy and fluorescent probes to confirm their identification and location inside the algal host.

1. Burr, F.A., and West, J.A. (1970). Light and electron microscope observations on the vegetative and reproductive structures of *Bryopsis hypnoides*. *Phycologia*, 9(1), 17-37.